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Ameliorative effects of Vitamin E and Selenium on haematological and serum biochemical parameters of growing pigs fed graded levels of crude oil contaminated diets

Okejim, Joy Anulichinyere and Osiagor, Wisdom Udochukwu*

Department of Agricultural Education, Federal College of Education (Technical), Omoku, Rivers State, Nigeria.

*Corresponding author. Email: osiagorwisdom@fcetomoku.edu.ng/osiagorwisdom@gmail.com; Co-author: okejimjoy@gmail.com

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ABSTRACT: This study was designed to evaluate the ameliorative effects of vitamin E alone and vitamin E plus selenium on haematological and serum biochemical parameters of growing pigs previously exposed to crude oil-contaminated diets. Thirty-six crossbred pigs (15.8±1.4 kg, 10–12 weeks) previously exposed to 0, 5, 10, or up till 15 g crude oil/kg feed for 4 weeks were assigned to three amelioration regimens for 4 weeks in 4×3 factorial completely randomized design (12 treatments, 3 replicates): no amelioration, vitamin E alone (200 mg/kg), and vitamin E + selenium (200 mg vitamin E + 5 mg selenium/kg), total 8 weeks plus 7 days acclimatization. Haematology (Hb, RBC, PCV, WBC) evaluated via cyanmethaemoglobin (540 nm), microhaematocrit, Neubauer haemocytometer and Leishman smears. Serum biochemistry (AST, ALT, ALP, GSH, GSH-Px, SOD, CAT, MDA) evaluated via Reitman and Frankel, Tietz, Beuter, Misra and Fridovich, Habig and TBARS methods. Data analyzed with SPSS v26.0. Pigs fed up till 15 g/kg without amelioration showed reduced Hb, RBC and PCV with 10.0 g/dL, 4.8×10^6/mm³ and 30% compared to control with 13.7 g/dL, 6.3×10^6/mm³ and 41.3% respectively, elevated WBC 9.2 vs 6.2, AST 56.5 vs 31.7, ALT 50.7 vs 39.7, ALP 57.0 vs 40.7 µ/L, GSH 2.5 vs 4.9 mg/dL, CAT 7.7 vs 13.3 U/mg and MDA 0.4 vs 0.3 nmol/ml ($p<0.05$). Vitamin E alone restored Hb to 13.0, PCV 39.0%, AST 51.5, GSH 3.2 and CAT 12.4 at up till 15 g/kg. Combined achieved complete restoration with Hb 13.5 vs 13.7, PCV 41.2% vs 41.3%, AST 45.0 vs 56.5, ALT 42.7 vs 50.7, ALP 44.0 vs 57.0, GSH 4.3 vs 2.5, CAT 12.4 vs 7.7 and MDA 0.3 vs 0.4. Vitamin E alone facilitates restoration up to 10 g/kg, combination facilitates complete amelioration even up to 15 g/kg.

Keywords: Crude oil, haematology, selenium, serum biochemistry, Vitamin E.

INTRODUCTION

Haematological and serum biochemical profiles constitute an integrated diagnostic window into the health status of pigs exposed to environmental toxicants, as blood transports oxygen, nutrients and waste while serum enzymes and antioxidant defences reflect organ integrity and redox balance (Jain, 1986; Kaneko *et al*., 2008). Unlike performance traits that manifest cumulative effects, alterations in haemoglobin (Hb), red blood cell (RBC), packed cell volume (PCV), white blood cell (WBC) counts and differentials, together with aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), reduced glutathione (GSH), glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), catalase (CAT) and malondialdehyde (MDA) provide early mechanistic evidence of haematotoxicity, hepatotoxicity and oxidative stress (Achuba and Osakwe, 2003; Sunmonu and Oloyede, 2006).

In the Niger Delta, where over 7,000 oil spill incidents have been reported since 1956 (UNEP, 2011; UNDP, 2006), pigs in free-range systems ingest crude oil via

contaminated forage, water and soil, predisposing to anaemia, immunosuppression, hepatic injury and redox imbalance. Crude oil is a complex mixture of aliphatic, alicyclic and polycyclic aromatic hydrocarbons (PAHs) and water-soluble fractions containing benzene, toluene, ethylbenzene and xylene (BTEX) and heavy metals (O'Clair and Rice, 1985; Williams *et al*., 1994). Benzene is a proven bone marrow toxin, causing hypoplastic anaemia through DNA adduct formation and inhibition of burst-forming units-erythroid (BFU-E) (Saita, 1974; Achuba and Osakwe, 2003). In rabbits, Ovuru and Ekweozor (2004) documented PCV decline 41% to 28% and Hb 14 to 9 g/dL within 14 days at 10 g/kg crude oil, while Achuba and Awhin (2009) reported RBC reduction and Heinz bodies. In goats, Ngodigha (2009) observed 15–20% PCV reduction, and in rats, Adegoke *et al*. (2012) reported Hb and PCV decline with elevated WBC. Recently, Oleforuh-Okoleh *et al*. (2023) and Oke *et al*. (2024) described hydrocarbon-induced oxidative damage to erythrocyte membranes and leukocytosis in poultry. Hepatotoxicity is evident as leakage of AST, ALT and ALP due to increased membrane permeability and cytoplasmic vacuolation and necrosis (Sunmonu and Oloyede, 2006, 2007; Ikanone *et al*., 2017; George and Sese, 2012a).

Oxidative stress is central to both haematotoxicity and biochemical derangements. Petroleum hydrocarbons are metabolised via cytochrome P450 to generate superoxide anion, hydrogen peroxide and hydroxyl radicals that attack polyunsaturated fatty acids in erythrocyte and hepatocyte membranes, generating MDA and depleting GSH, SOD and CAT (Halliwell, 1994; Onwurah, 1999). GSH is a master intracellular antioxidant existing in reduced (GSH) and oxidised (GSSG) forms, converting H_2O_2 to water via GPx, while SOD dismutates superoxide to H_2O_2 and CAT converts H_2O_2 to water and oxygen (Wu *et al*., 2004). Depletion leads to increased MDA and membrane leakage of AST, ALT, and ALP. Amelioration requires membrane and cytosolic protection. Vitamin E (α-tocopherol) is a lipid-soluble chain-breaking antioxidant in membranes, scavenging peroxyl radicals and preventing lipid peroxidation (Traber and Stevens, 2011; Shastak and Pelletier, 2025). Selenium is an essential trace element as a cofactor of GPx detoxifying H_2O_2 and organic peroxides (Rotruck *et al*., 1973; Tapiero *et al*., 2003). Their synergy has been shown in broilers under heat stress (Harsini *et al*., 2012), goats under arsenic toxicity (Roy and Roy, 2017) and transition cows (Salles *et al*., 2021; Ding *et al*., 2025), where combined supplementation increased GSH, SOD, CAT and reduced MDA more than individually. However, data in growing pigs exposed to graded crude oil levels evaluating both haematology and serum biochemistry with factorial analysis are scant. Therefore, this study was designed to evaluate comparative efficacy of supranutritional vitamin E alone (200 mg/kg) versus vitamin E plus selenium (200 mg + 5 mg/kg) across 0, 5, 10 and up to 15 g/kg histories, for a total of 8 weeks.

MATERIALS AND METHODS

Ethical approval

All animal handling and humane slaughter were approved by the Animal Ethics Committee, Rivers State University, Port Harcourt (Approval No: RSU/AEC/2021/015). Procedures were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and the Animal Research: Reporting of In Vivo Experiments (ARRIVE) 2.0 guidelines.

Experimental location and total duration

The study was conducted at the Piggery Unit of the Teaching and Research Farm, Federal College of Education (Technical), Omoku, Rivers State, Nigeria. The study area is located at approximately latitude 4°47′N and longitude 6°59′E and lies within the humid tropical zone. The area receives an average annual rainfall of approximately 2,300 mm, with ambient temperature ranging from 26 to 32°C and relative humidity of 70–90%. The experimental period lasted 8 weeks (56 days), comprising a 4-week contamination phase followed by a 4-week amelioration phase. Prior to commencement, animals underwent a 7-day acclimatisation period, resulting in a total duration of 63 days spent at the facility.

Source of crude oil and justification for graded levels

Bonny Light crude oil with American Petroleum Institute gravity of 35.4° was obtained from Nigerian Agip Oil Company, Obrikom Flow Station, Ogba/Egbema/Ndoni Local Government Area, Rivers State. The oil was weathered for 24 hours in a shallow pan under a shed at 27±2°C to allow evaporation of light and volatile fractions and to simulate environmental exposure after oil spillage, as described by Neff *et al*. (2000) and Ovuru and Ekweozor (2004). The weathered crude oil was thoroughly mixed with basal diet using a mechanical mixer for 10 minutes to ensure homogeneity. Graded levels of 0, 5, 10 and up to 15 g/kg were selected based on four criteria. First, environmental relevance: field surveys report 3.2–18.7 g/kg contamination in Niger Delta feeds (UNEP, 2011). Second, dose-response literature: Osiagor (2020) and Ovuru and Ekweozor (2004) reported dose-dependent haematotoxicity across 5–15 g/kg dietary crude oil exposure without mortality. Third, toxicity classification: 5 g/kg represents low chronic subclinical exposure, 10 g/kg moderate, and 15 g/kg high acute, causing anaemia and oxidative stress (Oke *et al*., 2024; Hong *et al*., 2024). Fourth, palatability: a preliminary trial showed >20 g/kg caused >40% feed refusal and 25 g/kg was rejected after 4 days in the pilot; hence, up to 15 g/kg was set as the upper limit.

Experimental animals, housing and management

Thirty-six clinically healthy crossbred growing pigs (Large White × Landrace, 18 males, 18 females, 10–12 weeks old, average weight 15.8±1.4 kg) were purchased from Cape Farms, Irete, Imo State and transported under humane conditions. The pigs were housed in a conventional open-sided tropical pig house with concrete flooring sloped at 5% for effective drainage. The house was partitioned into individual pens measuring 2.0 m × 1.2 m × 1.0 m per pig, with iron bar partitions, concrete feeding troughs 0.4 m wide and nipple drinkers for ad libitum water. Roofing was corrugated aluminium sheets at 3.5 m height to ensure natural ventilation, with a natural photoperiod of 12 hours light and 12 hours dark. Temperature and humidity were monitored daily using a hygro-thermometer, and biosecurity was maintained by foot dips containing Izal solution at 1:100 dilution at the entrance and restriction of visitors. Each pig served as a replicate and was housed individually to allow accurate feed intake measurement. Management practices observed included twice daily feeding at 08:00 h and 15:00 h, ad libitum clean water, daily pen washing and faeces removal, weekly disinfection with 2% Virkon® solution, daily health inspection for pale mucous membranes, weekly body weight recording, and gentle handling by the same personnel to minimise stress.

Pre-experimental veterinary care and acclimatisation

On arrival, all pigs underwent a 7-day acclimatisation period on basal diet *ad libitum*. Pre-experimental veterinary care was provided to stabilise animals. On day 1 of acclimatisation, long-acting oxytetracycline (Terramycin LA®) was administered intramuscularly at 20 mg/kg body weight. On day 2, ivermectin injection (Ivomec® 1%) was administered subcutaneously at 0.3 mg/kg for deworming. On day 3, animals were sprayed externally with Deltamethrin solution (Butox®) at 1 ml/L for ectoparasite control, and Vitalyte® multivitamin was provided in drinking water at 1 g/L for three consecutive days. Faecal samples were examined by flotation for helminth eggs and blood smears for haemoparasites. Only pigs negative for parasites and clinically healthy were enrolled.

Experimental diets

The basal diet was formulated to meet the nutrient requirements of growing pigs (20–50 kg) as recommended by the National Research Council (NRC, 2012). The diet consisted of maize 50.0%, soybean meal 44% crude protein 20.0%, palm kernel cake 10.0%, wheat offal 10.0%, fish meal 72% crude protein 3.0%, bone meal 2.5%, limestone 1.5%, salt 0.25%, vitamin-mineral premix 0.25%, L-Lysine 0.25% and DL-Methionine 0.25%, total 100.0%, containing 18.20% crude protein, metabolizable energy 2,950 kcal/kg, crude fibre 4.5%, ether extract 3.8%, calcium 0.85% and available phosphorus 0.45% as presented in Table 1. Three amelioration diets were prepared: basal alone without supplementation (no amelioration), basal + 200 mg vitamin E as DL-α-tocopheryl acetate per kg, and basal + 200 mg vitamin E + 5 mg selenium as sodium selenite per kg, based on supranutritional doses reported by Shastak and Pelletier (2025) and Reinoso-Maset *et al*. (2022). Diets were mixed biweekly to prevent oxidation.

Table 1. Gross and chemical composition of basal diet (as-fed basis).

Ingredient	Percentage (%)
Maize	50.0
Soybean meal (44% CP)	20.0
Palm kernel cake	10.0
Wheat offal	10.0
Fish meal (72% CP)	3.0
Bone meal	2.5
Limestone	1.5
Salt (NaCl)	0.25
Vitamin-mineral premix*	0.25
L-Lysine	0.25
DL-Methionine	0.25
Total	100.0

*Premix per kg: Vit A 10,000 IU, D3 2,000 IU, E 20 IU, K3 2 mg, B1 1.5 mg, B2 4 mg, B6 3 mg, B12 0.02 mg, Niacin 20 mg, Pantothenic acid 8 mg, Folic acid 0.8 mg, Biotin 0.05 mg, Choline 250 mg, Mn 80 mg, Zn 50 mg, Fe 60 mg, Cu 10 mg, I 1 mg, Co 0.2 mg, Se 0.1 mg basal. Calculated chemical composition: 18.20% CP, ME 2,950 kcal/kg, crude fibre 4.5%, ether extract 3.8%, Ca 0.85%, available P 0.45%.

Experimental design

Following the contamination phase, the experiment was arranged as a 4 × 3 factorial in a Completely Randomised Design (CRD). Factor A consisted of crude oil exposure history at four levels: 0, 5, 10, and 15 g/kg. Factor B comprised three amelioration treatments: no treatment, vitamin E supplementation, and a combination of vitamin E and selenium. This resulted in a total of twelve treatment combinations: T1–T3 (0 g/kg × three amelioration treatments), T4–T6 (5 g/kg × three treatments), T7–T9 (10 g/kg × three treatments), and T10–T12 (15 g/kg × three treatments). A total of 36 pigs were used in the study, with three replicates per treatment and one animal per replicate, each housed individually. Animals were randomly assigned to treatments using a simple randomisation method (drawing of lots) to ensure unbiased allocation.

Blood collection and haematological and serum biochemical analysis

Blood samples were collected on day 28 of the amelioration phase between 08:00 and 09:00 h prior to feeding by the same trained personnel to minimise diurnal variation. A total of 10 mL of blood was obtained from each pig via jugular venipuncture using a sterile 5 mL syringe fitted with an 18-gauge needle. Of this, 5 mL was transferred into labelled ethylenediaminetetraacetic acid (EDTA) tubes for haematological analysis; these samples were kept on ice and analysed within 2 hours of collection, while the remaining 5 mL was placed into heparinised tubes for serum biochemical analysis, centrifuged at 3,000 rpm for 10 minutes, and the serum stored at −20°C until analysis. Haemoglobin (Hb) concentration was determined using the cyanmethaemoglobin method with Drabkin's solution, and absorbance was read at 540 nm using a spectrophotometer (Spectrumlab S23A); Hb values were calculated using the formula Hb (g/dL) = (T × C × D) / (A × 1000), where T is the test absorbance, A is the standard absorbance, C is the standard concentration, and D is the dilution factor (Cheesbrough, 2000). Packed cell volume (PCV) was measured using the microhaematocrit method (Dacie and Lewis, 1991), in which capillary tubes were filled to three-quarters with EDTA blood, sealed with plasticine, centrifuged at 15,000 rpm for 5 minutes using a microhaematocrit centrifuge (Hawksley), and read using a microhaematocrit reader. Red blood cell (RBC) counts were determined using the Neubauer haemocytometer method (Jain, 1986) by diluting 0.02 mL of EDTA blood with 4 mL of formal citrate solution (prepared from 10 mL of 40% formalin in 1 L of 31.3 g/L trisodium citrate), loading the mixture into a counting chamber, and counting cells under a ×10 objective. White blood cell (WBC) counts were determined using an improved Neubauer haemocytometer with Turk's solution, by mixing 0.02 mL of blood with 0.38 mL of Turk's solution (1:20 dilution) and counting cells in four large squares (Jain, 1986). Differential leukocyte counts were obtained from Leishman-stained thin blood smears prepared using undiluted Leishman stain for 2 minutes followed by buffered water (pH 6.8) for 8 minutes, after which slides were washed, air-dried, and examined under oil immersion (×100), with 100 leukocytes counted per slide. Serum biochemical parameters were analyzed using standard methods: aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined using the colorimetric method of Reitman and Frankel as described by Ochei and Kolhatkar (2005), alkaline phosphatase (ALP) was measured according to Tietz (1976, 1995), catalase (CAT) activity was determined following Beutler (1982), superoxide dismutase (SOD) activity was measured using the epinephrine method of Misra and Fridovich (1972), reduced glutathione (GSH) and glutathione peroxidase (GSH-Px) were determined using the method of Habig *et al*. (1974), and malondialdehyde (MDA) levels were assessed using the thiobarbituric acid reactive substances (TBARS) method. All parameters were calculated from instrument readings using standard formulas and calibrated equipment rather than being measured directly.

Statistical analysis

All data were initially compiled in Microsoft Excel 2019 (Microsoft Corporation, Redmond, WA, USA) and subsequently analysed using IBM SPSS Statistics version 26.0 (IBM Corporation, Armonk, NY, USA) and GraphPad Prism version 9.0. A factorial analytical model was applied as follows:

$$Yijk = \mu + Ai + Bj + (AB)ij + eijk$$

Where Yijk is the observation, μ overall mean, Ai fixed effect of crude oil history (0, 5, 10, up to 15 g/kg), Bj fixed effect of amelioration (None, Vitamin E alone, Vitamin E + Selenium), (AB)ij interaction, eijk residual error.

The assumptions of normality and homogeneity of variance were tested using the Shapiro–Wilk test and Levene's test, respectively. When a significant interaction between crude oil history and amelioration treatment was detected ($p < 0.05$), treatment means for the twelve combinations were separated using Tukey's Honestly Significant Difference (HSD) test. For clarity of presentation, data within each amelioration treatment across crude oil exposure levels were also analysed using one-way analysis of variance; however, the primary conclusions were based on the factorial analysis. Statistical significance was declared at $p < 0.05$, and all results are presented as mean ± standard error of the mean (SEM).

RESULTS AND DISCUSSION

A factorial analysis (4 × 3) arranged in a Completely Randomised Design, comprising 12 treatments with three replicates of one pig each, revealed significant main effects of crude oil exposure history and amelioration regimen, as well as significant History × Amelioration interactions. These interactions were observed for haemoglobin (Hb; $p = 0.004$), packed cell volume (PCV; $p = 0.006$), white blood cell count (WBC; $p = 0.011$), aspartate aminotransferase (AST; $p = 0.002$), alanine aminotransferase (ALT; $p = 0.001$), alkaline phosphatase (ALP; $p = 0.001$), reduced glutathione (GSH; $p = 0.001$), catalase (CAT; $p = 0.001$), and malondialdehyde (MDA; $p = 0.001$). These results indicate that the extent of physiological recovery depended on both the level of prior crude oil contamination and the type of antioxidant treatment administered. For clarity, the simple effects of crude oil history within each amelioration regimen are

Table 2. Effects of no amelioration on haematological parameters of growing pigs with different crude oil histories (up to 15 g/kg, 28-day amelioration).

Parameter	0 g/kg (Control)	5 g/kg	10 g/kg	15 g/kg	SEM	p-value
Hb (g/dL)	13.7[a]	13.5[a]	13.3[a]	10.0[b]	0.5	0.001
RBC (×10⁶/mm³)	6.3[a]	6.1[a]	5.9[a]	4.8[b]	0.2	0.001
PCV (%)	41.3[a]	40.7[a]	40.7[a]	30.0[b]	1.5	0.001
WBC (×10⁶/mm³)	6.2[a]	6.6[a]	6.4[a]	9.2[b]	0.2	0.001
Neutrophils (%)	46.7[a]	36.7[b]	38.0[b]	48.0[a]	1.0	0.042
Lymphocytes (%)	50.7[a]	62.0[b]	59.3[b]	50.7[a]	1.2	0.038
Monocytes (%)	2.0[a]	0.3[b]	0.7[b]	0.3[b]	0.3	0.008

Hb, RBC, PCV reduced by 10.0 g/dL, 4.8×10⁶/mm³ and 30%, respectively, compared to the control with 13.7 g/dL, 6.3×10⁶/mm³ and 41.3%, respectively, at up to 15 g/kg ($p<0.05$). Reference values: PCV 32–50%, Hb 10–16 g/dL, RBC 5–8×10⁶/µL (Jain, 1986; MSD, 2024). [ab] Means differ ($p<0.05$). SEM = Standard Error of the Mean.

Table 3. Effects of Vitamin E alone (200 mg/kg) on haematological parameters.

Parameter	0 g/kg (Control)	5 g/kg	10 g/kg	15 g/kg	SEM	p-value
Hb (g/dL)	13.7[a]	15.8[b]	15.0[b]	13.0[a]	0.5	0.002
RBC (×10⁶/mm³)	6.3	6.8	6.5	6.0	0.2	0.124
PCV (%)	41.3	42.0	40.0	39.0	1.5	0.213
WBC (×10⁶/mm³)	6.2[a]	6.4[a]	6.5[a]	8.7[b]	0.2	0.001
Neutrophils (%)	46.7[a]	42.7[b]	48.3[a]	45.0[a]	1.0	0.032
Lymphocytes (%)	50.6[a]	54.3[a]	50.3[a]	54.7[b]	1.2	0.041
Monocytes (%)	2.0[a]	1.7[a]	0.3[b]	0.0[b]	0.3	0.006

Vitamin E alone fully restored Hb, RBC, PCV to within reference values up to 10 g/kg and at up till 15 g/kg (13.0 g/dL, 6.0×10⁶/mm³, 39.0% within 10–16, 5–8, 32–50%). [ab] Means differ ($p<0.05$).

presented in Tables 2-7. The control values recorded in this study fell within the established physiological reference ranges for healthy growing pigs, thereby providing a valid baseline for assessing anaemia and other alterations.

Combined vitamin E and selenium (VE+Se) supplementation markedly improved haematological parameters, restoring PCV to 41.2% at contamination levels up to 15 g/kg, compared with 30.0% in the non-ameliorated group and 39.0% with vitamin E alone. Similarly, Hb (13.5 g/dL vs 10.0 g/dL) and RBC (5.8 ×10⁶/µL vs 4.8 ×10⁶/µL) values were restored to within normal reference ranges. The combined treatment also more effectively reduced WBC counts (8.4 vs 9.2 ×10³/µL), indicating improved immune status relative to the non-ameliorated group.

In contrast, pigs in the non-ameliorated group exhibited elevated liver enzyme activities, with AST (56.5 U/L), ALT (50.7 U/L), and ALP (57.0 U/L) compared to control values of 31.7, 39.7, and 40.7 U/L, respectively. Markers of oxidative stress were also adversely affected, as shown by reduced GSH (2.5 vs 4.9) and CAT (7.7 vs 13.3), alongside increased MDA levels (0.4 vs 0.3) at contamination levels up to 15 g/kg ($p < 0.05$). These findings indicate pronounced hepatic dysfunction and oxidative damage in the absence of antioxidant intervention.

To contextualise these findings, the haematological values obtained in this study were compared with established physiological reference ranges for growing pigs. According to classical texts such as *Schalm's Veterinary Haematology* (Jain, 1986) and *Practical Haematology* (Dacie and Lewis, 1991), normal ranges for pigs weighing 20–50 kg include PCV (32–50%), Hb (10–16 g/dL), RBC (5–8 × 10⁶/µL), and WBC (11–22 × 10³/µL), with differential counts of neutrophils (28–47%), lymphocytes (39–62%), and monocytes (2–10%). These values are consistent with those reported in the MSD Veterinary Manual (2024), which provides comparable ranges for porcine Hb (100–160 g/L), PCV (0.32–0.50 L/L), and RBC (5.0–8.0 × 10¹²/L).

More recent population-based studies further support these reference intervals, although slight variations exist due to differences in age, management, and physiological status. For instance, Perri *et al*. (2017) reported values in Ontario commercial pigs close to weaning as RBC (4.8–7.3 × 10¹²/L), Hb (93–136 g/L), PCV (0.30–0.50 L/L), and WBC (6.0–21.7 × 10⁹/L), while Zhang *et al*. (2022) documented ranges in healthy nursery pigs in China as RBC (5.62–7.84 × 10¹²/L), Hb (92.2–135.2 g/L), PCV (32.11–47.85%), and WBC (7.18–24.52 × 10⁹/L). Broader ranges have also been reported in adult pigs, including sows (Boulbria *et al*., 2021), reflecting physiological and reproductive influences.

The consistency between these established reference values and the control group observed in this study

Table 4. Effects of Vitamin E + Selenium (200 mg + 5 mg/kg) on haematological parameters.

Parameter	0 g/kg (Control)	5 g/kg	10 g/kg	15 g/kg	SEM	p-value
Hb (g/dL)	13.7[a]	16.0[b]	16.2[b]	13.5[a]	0.5	0.001
RBC (×10⁶/mm³)	6.3	6.6	6.3	5.8	0.2	0.187
PCV (%)	41.3	45.0	43.5	41.2	1.5	0.092
WBC (×10⁶/mm³)	6.2[a]	6.4[a]	6.0[a]	8.4[b]	0.2	0.001
Neutrophils (%)	46.7[a]	50.6[b]	52.3[b]	53.0[b]	1.0	0.004
Lymphocytes (%)	50.7[a]	48.7[a]	45.1[b]	46.7[b]	1.2	0.006
Monocytes (%)	2.0[a]	0.0[b]	1.3[a]	3.3[c]	0.3	0.001

Combined VE+Se restored PCV to 41.2% at up till 15 g/kg vs 30.0% non-ameliorated and 39.0% VE alone, Hb 13.5 vs 10.0, RBC 5.8 vs 4.8. [abc] Means differ ($p<0.05$).

Table 5. Effects of no amelioration on serum biochemical parameters (liver enzymes, antioxidants and MDA).

Parameter	0 g/kg (Control)	5 g/kg	10 g/kg	15 g/kg	SEM	p-value
AST (µ/L)	31.7[a]	32.5[a]	33.0[a]	56.5[b]	1.2	0.002
ALT (µ/L)	39.7[a]	38.0[a]	42.0[a]	50.7[b]	1.4	0.001
ALP (µ/L)	40.7[a]	44.0[a]	44.2[a]	57.0[b]	1.2	0.001
GSH (mg/dL)	4.9[a]	4.9[a]	4.8[b]	2.5[c]	0.01	0.001
GSH-Px (nmol/dL)	2.5[a]	2.8[a]	2.8[a]	3.3[b]	0.10	0.001
SOD (U/mg)	0.2[a]	0.2[a]	0.2[a]	0.1[b]	0.01	0.002
CAT (IU/mg)	13.3[a]	13.5[a]	13.7[a]	7.7[b]	0.30	0.001
MDA (nmol/ml)	0.3[a]	0.3[a]	0.3[a]	0.4[b]	0.03	0.001

Elevated AST with 56.5 µ/L, ALT with 50.7 and ALP with 57.0 vs control with 31.7, 39.7 and 40.7 respectively at up till 15 g/kg ($p<0.05$). [abc] Means differ.

validates the baseline measurements and strengthens the interpretation of treatment effects. Consequently, the reductions in haematological parameters recorded in crude oil–exposed, non-ameliorated pigs can be reliably attributed to toxicological stress rather than normal biological variation. These observations further support earlier reports linking petroleum hydrocarbon exposure to haematological suppression and oxidative stress in animals (Ovuru and Ekweozor, 2004; Achuba and Osakwe, 2003), thereby reinforcing the biological relevance of the present findings.

The control group (0 g/kg crude oil) in this study exhibited values within established physiological reference ranges, thereby providing a reliable baseline for identifying pathological changes. Haematological parameters showed that haemoglobin (Hb, 13.7 g/dL) fell within the standard range of 10–16 g/dL (Jain, 1986; MSD, 2024) and also within 9.3–13.6 g/dL reported by Perri *et al*. (2017), although slightly above the upper limit of 13.52 g/dL for nursery pigs (Zhang *et al*., 2022), but still physiologically normal. Red blood cell count (RBC, 6.3 × 10⁶/mm³) was within the range of 5–8 × 10⁶/mm³ and consistent with values reported by Perri *et al*. (2017) and Zhang *et al*. (2022). Packed cell volume (PCV, 41.3%) also fell within the normal range of 32–50% and was consistent with the 32.11–47.85% reported by Zhang *et al*. (2022). White blood cell count (WBC, 6.2 × 10⁹/L) was within the range of 6.0–21.7 × 10⁹/L (Perri *et al*., 2017), though slightly below the lower limit of 7.18 × 10⁹/L reported by Zhang *et al*. (2022), yet still within acceptable physiological limits. Differential counts were similarly within normal ranges: neutrophils (46.7%), lymphocytes (50.7%), and monocytes (2.0%) corresponded with established reference intervals (Jain, 1986; Zhang *et al*., 2022). Serum biochemical parameters in the control group—AST (31.7 U/L), ALT (39.7 U/L), and ALP (40.7 U/L)—also fell within normal ranges reported by Kaneko *et al*. (2008). Antioxidant indices (GSH, CAT, and MDA) were within expected physiological limits, confirming that the control animals were healthy and validating the experimental baseline.

In contrast, pigs fed diets containing up to 15 g/kg crude oil without amelioration exhibited clear evidence of anaemia and hepatocellular injury, rather than merely relative reductions compared to the control. Haemoglobin (10.0 g/dL), RBC (4.8 × 10⁶/mm³), and PCV (30%) were markedly reduced compared to control values (13.7 g/dL, 6.3 × 10⁶/mm³, and 41.3%, respectively). These values fall at or below established lower reference limits (Jain, 1986; MSD, 2024), fulfilling the classical diagnostic criteria for anaemia (Jain, 1986; Dacie and Lewis, 1991). Similar findings have been reported in other species exposed to petroleum hydrocarbons, including rabbits (Ovuru and Ekweozor, 2004) and goats (Ngodigha, 2009), as well as poultry (Oleforuh-Okoleh *et al*., 2023), confirming anaemia as a consistent indicator of hydrocarbon toxicity. Although WBC (9.2 × 10⁹/L) remained within the normal range, it was significantly elevated relative to the control (6.2 × 10⁹/L), representing a 48.4% increase ($p = 0.001$) and

Table 6. Effects of Vitamin E alone (200 mg/kg) on serum biochemical parameters.

Parameter	0 g/kg (Control)	5 g/kg	10 g/kg	15 g/kg	SEM	p-value
AST (µ/L)	31.7[a]	32.0[a]	32.7[a]	51.5[b]	1.2	0.002
ALT (µ/L)	39.7	42.0	38.7	43.9	1.4	0.345
ALP (µ/L)	40.7[a]	42.6[a]	43.5[a]	50.0[b]	1.2	0.003
GSH (mg/dL)	4.9[a]	4.9[b]	4.9[b]	3.2[b]	0.01	0.001
GSH-Px (nmol/dL)	2.5	2.6	2.7	2.8	0.10	0.234
SOD (U/mg)	0.2	0.2	0.2	0.2	0.01	0.456
CAT (IU/mg)	13.3[a]	13.5[a]	13.7[a]	12.4[b]	0.30	0.002
MDA (nmol/ml)	0.3[a]	0.3[a]	0.3[a]	0.4[b]	0.03	0.001

Vitamin E alone reduced AST to 51.5, ALT to 43.9, ALP to 50.0 at up till 15 g/kg and restored GSH to 3.2, CAT to 12.4. [ab] Means differ ($p<0.05$).

Table 7. Effects of Vitamin E + Selenium (200 mg + 5 mg/kg) on serum biochemical parameters.

Parameter	0 g/kg (Control)	5 g/kg	10 g/kg	15 g/kg	SEM	p-value
AST (µ/L)	31.7[a]	31.5[a]	32.0[a]	45.0[b]	1.2	0.002
ALT (µ/L)	39.7	38.0	36.3	42.7	1.4	0.234
ALP (µ/L)	40.7	41.0	42.3	44.0	1.2	0.345
GSH (mg/dL)	4.9[a]	4.9[a]	4.8[a]	4.3[b]	0.01	0.001
GSH-Px (nmol/dL)	2.5	2.8	2.8	2.8	0.10	0.123
SOD (U/mg)	0.2	0.2	0.2	0.2	0.01	0.567
CAT (IU/mg)	13.3	13.8	13.6	12.4	0.30	0.234
MDA (nmol/ml)	0.3	0.3	0.3	0.3	0.03	0.678

Combined VE+Se restored AST 45.0 vs 56.5, ALT 42.7 vs 50.7, ALP 44.0 vs 57.0, GSH 4.3 vs 2.5, CAT 12.4 vs 7.7 and MDA 0.3 vs 0.4. [ab] Means differ ($p<0.05$).

indicating inflammatory stress (Dede *et al*., 2002; Oke *et al*., 2024). Liver enzyme activities were also elevated, with AST (56.5 U/L), ALT (50.7 U/L), and ALP (57.0 U/L) exceeding control values, indicating hepatocellular damage and increased membrane permeability (George and Sese, 2012a; Ikanone *et al*., 2017). Furthermore, oxidative stress was evident, as shown by reduced GSH (2.5 vs 4.9 mg/dL) and CAT (7.7 vs 13.3 IU/mg), alongside increased MDA levels (0.4 vs 0.3). These findings confirm that crude oil exposure induces anaemia, liver dysfunction, and oxidative stress when values deviate beyond physiological reference limits.

Vitamin E supplementation (200 mg/kg) improved both haematological and biochemical parameters, achieving near-complete restoration at contamination levels up to 10 g/kg and partial recovery at 15 g/kg. As a lipid-soluble, chain-breaking antioxidant located within cell membranes, vitamin E scavenges peroxyl radicals and prevents lipid peroxidation of erythrocyte and hepatocyte membranes (Traber and Stevens, 2011; Shastak and Pelletier, 2025). This action helps maintain membrane integrity, preserve erythrocyte deformability, and reduce enzyme leakage (Achuba and Awhin, 2009; Ognjanovic *et al*., 2003). However, at higher contamination levels (up to 15 g/kg), oxidative stress exceeds the protective capacity of vitamin E alone. In such cases, selenium becomes essential as a cofactor for glutathione peroxidase (GSH-Px), which detoxifies hydrogen peroxide in the cytosol, preserves reduced glutathione, enhances vitamin E activity, and activates the Nrf2 antioxidant pathway (Rotruck *et al*., 1973; Machlin, 1991; Reinoso-Maset *et al*., 2022; Wang *et al*., 2021). The combined supplementation of vitamin E and selenium therefore provides a complementary defense system, significantly reducing MDA (0.4 to 0.3 nmol/mL), restoring GSH (2.5 to 4.3 mg/dL) and CAT (7.7 to 12.4 IU/mg), improving PCV (41.2% vs 30.0% in non-ameliorated and 39.0% with vitamin E alone), and lowering AST (45.0 vs 56.5 U/L; compared to 51.5 U/L with vitamin E alone). These results demonstrate superior efficacy of the combined treatment, consistent with previous findings (Harsini *et al*., 2012; Aslam *et al*., 2010). Overall, the dose-dependent response indicates that vitamin E alone is effective at lower contamination levels (≤10 g/kg), whereas combined vitamin E and selenium is required for optimal protection at higher contamination levels (up to 15 g/kg), such as those encountered near oil spill sites.

Conclusion

Exposure to crude oil at levels up to 15 g/kg for four weeks caused significant haematological and serum biochemical

alterations in growing pigs. These effects were evident as reductions in haemoglobin (10.0 g/dL), red blood cell count (4.8 × 10^6/mm^3), and packed cell volume (30%) compared with control values (13.7 g/dL, 6.3 × 10^6/mm^3, and 41.3%, respectively), alongside an increased white blood cell count (9.2 vs 6.2 × 10^9/L), indicating an inflammatory response. Liver enzyme activities were elevated (AST 56.5 U/L, ALT 50.7 U/L, ALP 57.0 U/L) relative to controls (31.7, 39.7, and 40.7 U/L), while oxidative stress was demonstrated by reduced glutathione (2.5 vs 4.9 mg/dL) and catalase (7.7 vs 13.3 IU/mg), and increased malondialdehyde (0.4 vs 0.3 nmol/mL). These deviations from established physiological reference ranges (PCV 32–50%, Hb 10–16 g/dL, RBC 5–8 × 10^6/mm^3) confirm the presence of anaemia, hepatocellular injury, and oxidative imbalance. Vitamin E supplementation alone restored haematological parameters and partially improved biochemical indices at contamination levels up to 10 g/kg, whereas combined supplementation with vitamin E and selenium achieved near-complete recovery even at 15 g/kg, restoring haematological values close to control levels and normalising liver enzyme activities and antioxidant status. Overall, vitamin E alone is effective at lower contamination levels (≤10 g/kg), while the combined use of vitamin E and selenium provides superior protection and complete amelioration at higher contamination levels (≤15 g/kg).

Recommendations

1. Regular haematological and serum biochemical assessments should be conducted as early diagnostic tools in pigs exposed to crude oil contamination. Indicators such as PCV < 32%, Hb < 10 g/dL, and AST > 50 U/L should be considered evidence of toxicity. For management, vitamin E supplementation at 200 mg/kg feed is recommended for contamination levels up to 10 g/kg, while a combination of vitamin E (200 mg/kg) and selenium (5 mg/kg) should be used at levels up to 15 g/kg.
2. Feed manufacturers operating in high-risk or oil-contaminated regions should reformulate premixes to include supranutritional levels of antioxidants, particularly vitamin E and selenium, to mitigate the effects of environmental toxins.
3. Further studies should investigate the effects of crude oil toxicity on bone marrow histology and erythropoietin regulation, evaluate the efficacy of organic selenium sources, explore the role of the Nrf2 antioxidant pathway, and assess the cost–benefit implications of antioxidant supplementation under field conditions.

CONFLICT OF INTEREST

There is no conflict of interest involved in writing the paper.

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